

Independence Day for IgA

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IgA is induced through T-cell-dependent and -independent pathways. In this issue, [Bunker et al. \(2015\)](#) now show that the T-cell-independent pathway is sufficient to coat most small intestinal microbes specifically, and [Fransen et al. \(2015\)](#) find that IgA coating promotes uptake of microbes into Peyer's patches and drives further induction in a positive-feedback loop.

"But my dear Sebastian ... you can't believe things because they're a lovely idea."

"But I do. That's how I believe."

—Evelyn Waugh (*Brideshead Revisited*)

That immunoglobulin A (IgA) generally protects the intestinal mucosa from the challenges of non-pathogenic members of the intestinal microbiota is commonly stated in immunology texts, with remarkably little evidence to show exactly how this works or how extensively microbial taxa need to be targeted. Two papers in the current issue of *Immunity* now provide some clarification of these issues ([Bunker et al., 2015](#); [Fransen et al., 2015](#)).

Previous studies have looked at how IgA controls commensal bacteria. Mice secreting monoclonal IgA from a subcutaneous hybridoma directed against a single intestinal bacterium (*Bacteroides thetaiotaomicron*) in RAG-deficient mice show reduced mucosal innate immune activation and the bacteria themselves have less oxidative stress ([Peterson et al., 2007](#)). Specific secretory (S)IgA responses to single non-pathogenic members of the microbiota generated through T-cell-dependent or -independent pathways ([Macpherson et al., 2000](#)) limit penetration of high doses of intestinal bacteria into deeper host tissues.

Such highly simplified models make the assumption that a single microbe can be taken as a first approximation of what is happening in a complex microbiota containing hundreds of different taxa. Because it is hard to standardize such complex microbiota even across cages in a vivarium, the approach has often been to compare different mouse strains with roughly equivalent diverse intestinal

microbial consortia, with or without host defects in IgA induction or secretion. For example, the failure of IgA class switch recombination due to a mutation in AID (activation-induced cytidine deaminase, G23S) reveals increased mucosal immune induction through hyperplasia of intestinal lymphoid structures and microbiota composition disturbances ([Wei et al., 2011](#)), whereas targeted deletion of the polymeric Ig receptor protein (plgR, responsible for IgA secretion across epithelial cells) results in mucosal damage and leakage of serum proteins into the intestine ([Johansen et al., 1999](#)).

Experimental results with these different "bottom-up" (simple gnotobiotic) and "top-down" (host-strain combination) approaches require the caveat that there is a large body of evidence that isolated deficiency of the IgA isotype can be accommodated in both mice and humans with remarkably few ill effects—probably mainly because polymeric IgM secretion can compensate for the lack of SIgA. However, loss of all Ig isotypes (in human common variable immunodeficiency) frequently leads to serious intestinal failure.

It is a truism that the hundreds of microbial taxa in an intestinal microbiota encompass a hugely diverse community with differences in metabolism, inter-microbial interactions, niches, and potential pathogenicity. There are good examples of different IgA responses to particular microbes. The earliest studies of specific IgA were done with cholera toxin as an immunogen that has a specific and highly T-cell-dependent IgA response—which protects against the secretory effects of toxin challenge ([Lycke et al., 1987](#)). More recently, segmented filamentous bacteria (SFB, *Candidatus arthromitus*) were shown not only to induce strong

IgA responses, but also to overgrow in the intestine if IgA was absent ([Suzuki et al., 2004](#)). Of course, these might be considered rather special cases: cholera toxin because of its dramatic G protein-coupled activation of adenylate cyclase signaling and SFB because of its intimate attachment to intestinal epithelial cells in the ileum. Nevertheless, we need to ask more generally how we should understand the significance and mechanisms of different IgA responses against diverse individual taxa within complex microbiotas.

It has long been known that, once IgA has been transported across the epithelium into the intestinal lumen, it coats the surfaces of intestinal bacteria. Recently, substantial progress on the significance of IgA binding to particular taxa was made by flow-cytometric sorting of microbes in complex large intestinal and fecal consortia according to the extent of their individual IgA coating ([Figure 1](#)). The taxa comprising those subsets of microbes coated with IgA in vivo (and those that have not been IgA coated) were then identified through amplicon sequencing of the genes for 16S ribosomal RNA ([Palm et al., 2014](#)). This IgA-seq approach shows (1) that there are a range of taxa that are preferentially coated with IgA in vivo; (2) some microbes require the T-cell-dependent IgA induction pathway whereas others are coated with T-cell-independent IgA; and (3) if IgA-coated microbes (derived from either laboratory mice or human patients with inflammatory bowel disease) are transferred into germ-free mice, it is easier to induce experimental colitis than in animals where IgA-uncoated microbes are transferred. The functional importance of IgA coating in vivo has also been studied in children with kwashiorkor protein-energy malnutrition, where fecal IgA-coated

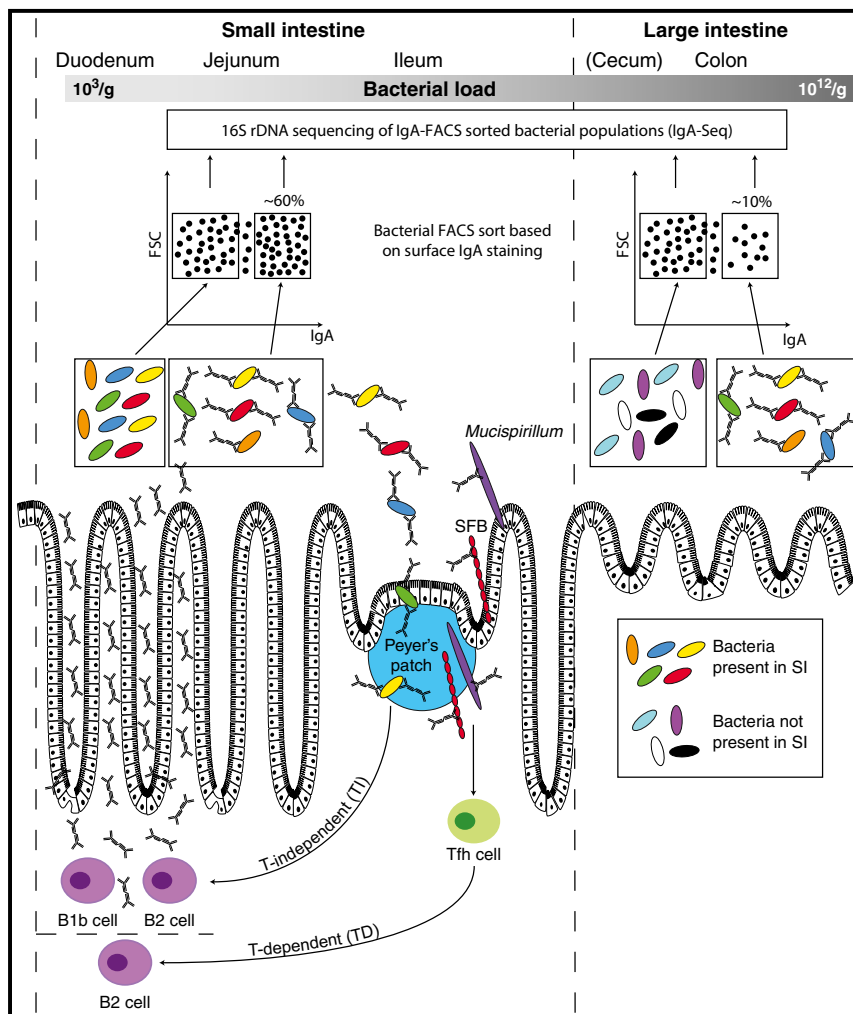


Figure 1. Microbial Coating with IgA along the Intestinal Tract

IgA induction occurs in the small intestine either through a T-cell-independent (TI) or a T-cell-dependent (TD) pathway. 16S amplicon sequencing of FACS-sorted IgA-coated compared with IgA-uncoated bacterial populations from the small intestine and colon reveal that commensal-specific IgA induced in the small intestine can coat bacteria present in the small intestine. In contrast, in the colon only bacteria that are also present in the small intestine are coated. Bacterial IgA coating assists uptake into lymphoid structures and induction of further IgA.

consortia from affected humans can trigger intestinal inflammation and dysfunction when transferred into germ-free mice (Kau et al., 2015). We can conclude that the IgA responses of both mice and humans are identifying sets of potentially pathogenic microbes and that these organisms are then coated with IgA in the intestinal lumen. Where in the intestinal tract does this happen and what are the cellular requirements for the response?

In this issue of *Immunity*, Bunker et al. (2015) have addressed the questions of how and where microbe-specific IgA is induced by combining IgA-seq and host strain combination techniques in diversely

colonized mice. They find that IgA responses to most taxa in the microbiota can be satisfactorily induced in mice that are doubly deficient in the beta and delta T cell receptor (TCR) chains (thus lacking cognate T cell help) or in mice conditionally deficient for BCL-6 (lacking follicular helper T cells and germinal center formation). The presence of T cell help makes only a small difference to the anti-bacterial IgA repertoire, although it does considerably amplify the magnitude of the response. Because it was possible to substitute an engineered IgG1 Fc for the IgA Fc in recombinant monoclonal IgA antibodies from the

Tcrb^{-/-}*d*^{-/-} strain without compromising anti-bacterial specificity, binding is probably occurring via the conventional Fab site. There are some exceptions to this rather promiscuous expendability of cognate T cell help: IgA induction to SFB and *Mucispirillum*, both of which interact intimately with the epithelium of the terminal ileum, require obligatory TCR signaling (Figure 1).

IgA-seq has also given some new insights into the dominant site of microbial IgA induction. Whereas IgA coated a proportion of most bacterial taxa in the duodenum, many colonic taxa were entirely IgA negative (Bunker et al., 2015). This highlights the importance of IgA induction in the small intestine—exactly where the host must limit microbial effects on the mucosa without the aid of a developed double mucus layer, to compete effectively with its microbiota for nutrition.

What about the old issue that isolated IgA deficiency has a very weak phenotype in either mice or humans? A further important result of the work by Bunker et al. (2015) has been to show that in the absence of IgA—in this case because of global AID deficiency, which abolishes both class switch recombination and somatic hypermutation to improve binding affinities—IgM substituted for IgA with corresponding binding to taxa that are normally preferentially IgA bound. This reinforces the view that secreted IgM can partially compensate for IgA deficiency (Bunker et al., 2015).

These studies move bacterial IgA binding from a tool that discriminates taxa within the microbiota with potential pathogenicity to showing that the binding repertoire is largely unbiased in the small intestine, whether or not induction proceeds by T-cell-dependent or T-cell-independent means.

Just as one species of bacteria cannot be entirely representative of the IgA response, neither can one host genetic background. In a second paper in this issue of *Immunity*, Franssen et al. (2015) have shown that, compared with C57BL/6 mice that are most widely studied, BALB/c mice have higher spontaneous IgA secretion of natural antibodies, even when they are germ free. These natural antibodies promote uptake of coated bacteria into the Peyer's patches (of the small intestine) and induction of a further IgA response in an apparent

positive feedback loop (Figure 1). The notion that natural specificities can have an important effect potentially fits with earlier experiments on the importance of IgA in promoting mucosal antigen uptake (Corthésy, 2007) and the outcome of B cell subset transfer experiments in the paper from Bunker et al. (2015), where they concluded that B1 cells (but the B1b subset rather than the B1a subset normally associated with IgM natural specificities) contribute to the T-cell-independent IgA response.

Appropriately for papers in *Immunity*, we are looking at the microbial dramatis personae—who is there—determined by the IgA response in different mouse strains and intestinal niches. Together they emphasize that the germline-encoded Ig repertoire is likely to be very important as a starting point for host-microbial mutualism, notwithstanding clear evidence for substantial later T-cell-dependent diversification. Although we can infer metabolic signatures of those

microbial taxa that are preferentially IgA bound from their genomes, we still need better information on how these organisms actually respond to life in the intestine with the challenges of nutrient and electron acceptor limitations, bile acids, and the consequences of quorum sensing potentially driving symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. We've come around full circle, to a (as yet incompletely justified) belief in the lovely idea that IgA (or IgM) actively shapes these processes for the mutual benefit of the host and microbiota alike.

REFERENCES

- Bunker, J.J., Flynn, T.M., Koval, J.C., Shaw, D.G., Meisel, M., McDonald, B.D., Ishizuka, I.E., Dent, A.L., Wilson, P.C., Jabri, B., et al. (2015). *Immunity* 43, this issue, 541–553.
- Corthésy, B. (2007). *J. Immunol.* 178, 27–32.
- Fransen, F., Zagato, E., Mazzini, E., Fosso, B., Manzari, C., El Aidy, S., Chiavelli, A., D'Erchia, A.M., Sethi, M.K., Pabst, O., et al. (2015). *Immunity* 43, this issue, 527–540.
- Johansen, F.E., Pekna, M., Norderhaug, I.N., Haneberg, B., Hietala, M.A., Krajci, P., Betsholtz, C., and Brandtzaeg, P. (1999). *J. Exp. Med.* 190, 915–922.
- Kau, A.L., Planer, J.D., Liu, J., Rao, S., Yatsunenkov, T., Trehan, I., Manary, M.J., Liu, T.C., Stappenbeck, T.S., Maleta, K.M., et al. (2015). *Sci. Transl. Med.* 7, 276ra24.
- Lycke, N., Eriksen, L., and Holmgren, J. (1987). *Scand. J. Immunol.* 25, 413–419.
- Macpherson, A.J., Gatto, D., Sainsbury, E., Harri-man, G.R., Hengartner, H., and Zinkernagel, R.M. (2000). *Science* 288, 2222–2226.
- Palm, N.W., de Zoete, M.R., Cullen, T.W., Barry, N.A., Stefanowski, J., Hao, L., Degnan, P.H., Hu, J., Peter, I., Zhang, W., et al. (2014). *Cell* 158, 1000–1010.
- Peterson, D.A., McNulty, N.P., Guruge, J.L., and Gordon, J.I. (2007). *Cell Host Microbe* 2, 328–339.
- Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T., and Fagarasan, S. (2004). *Proc. Natl. Acad. Sci. USA* 101, 1981–1986.
- Wei, M., Shinkura, R., Doi, Y., Maruya, M., Fagarasan, S., and Honjo, T. (2011). *Nat. Immunol.* 12, 264–270.

Close Encounters of the Tertiary Kind

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Elucidating the function of tumor-infiltrating regulatory T (Treg) cells has been difficult. In this issue of *Immunity*, Joshi et al. (2015) demonstrate that Treg cells associated with murine lung cancers are found within tertiary lymphoid structures and actively restrain effector T cells at the tumor site.

The recent clinical success of immune-based therapies in multiple human cancer types demonstrates that the immune system can be manipulated for clinical benefit (Page et al., 2014). This success has triggered increased activity aimed at understanding the inhibitory mechanisms restricting anti-tumor immunity in order to optimize clinical efficacy and broaden the spectrum of patients who benefit from these therapies. Front and center in this line of inquiry are CD4⁺Foxp3⁺ regulatory T (Treg) cells, which are critical for the suppression of immune responses and the maintenance

of immune homeostasis. Treg cells are present at elevated densities in many human and murine cancers, suggesting that these cells might play functional roles in the tumor microenvironment. Epidemiological data from human cancer studies reveal that for some cancer types, a high density of Treg cells within tumor lesions is predictive of poor outcome, whereas in other cancers, the opposite effect is observed (deLeeuw et al., 2012). Therefore, although it is widely hypothesized that tumor-infiltrating Treg cells promote tumor development by shielding tumors from immune attack,

it is equally plausible that in some cancer contexts, Treg cells might restrict tumor development by other mechanisms, such as suppressing tumor-promoting inflammation. These complexities highlight the importance of elucidating the functional roles of tumor-infiltrating Treg cells in different cancer types. However, such efforts have been hampered by a lack of approaches in tractable animal models that enable the selective ablation of Treg cells within tumor lesions while leaving Treg cells distributed elsewhere in the body untouched. In this issue of *Immunity*, Joshi et al. (2015)